Supplemental Protocols

Protocol 1: Recipes for chemical solutions

Longmire's Solution (Longmire, et al., 1991, 1997; Renshaw, et al., 2015; Williams, et al., 2016)

Recipe is for total volume: **500 mL**

Stock Chemical Name	[Stock]	[Final]	Volume/Amount
Tris Base pH 8	1M	0.1M	50 mL
Disodium-EDTA pH 8	0.5M	0.1M	100 mL
Sodium chloride (NaCl)	5M	0.01M	1 mL
Sodium dodecyl sulfate (SDS)	10%	0.5%	25 mL
Water			324 mL

Recipe notes:

- Sodium azide can be added to prevent microbial growth (Williams, et al., 2016)
- Keep all waste in a properly labelled Hazardous Waste container.

Lysis Buffer I (Lever, et al., 2015)

Recipe is for total volume: **250 mL**

Stock Chemical Name	[Stock]	[Final]	Volume/Amount
Tris Base pH 8	1M	0.03M	7.5 mL
Disodium-EDTA pH 8	0.5M	0.03M	15 mL
Guanidium hydrochloride	1M	0.8M	200 mL
Triton X-100	10%	0.5%	12.5 mL
Water			15 mL

Recipe notes:

- Adjust final solution to pH [10.0] using NaOH pellets ($n = 2$) + ~1mL [1M] NaOH
- Keep all waste in a properly labelled Hazardous Waste container.

PEG8000-NaCl Precipitation Solution (Cseke, et al., 2011 "Handbook of Molecular and Cellular Methods in Biology and Medicine, Third Edition"; Lever, et al., 2015)

Recipe is for total volume: **100 mL**

Stock Chemical Name	[Stock]	[Final]	Volume/Amount
Polyethylene glycol 8000	100%	30%	30 g
Sodium chloride (NaCl)	5M	1.6M	32 mL
Water			68 mL

Recipe notes:

- No pH adjustment required (unadjusted solution is acidic).
- Keep all waste in a properly labelled Hazardous Waste container.

Protocol 2: Preparation of DNAse-treated glycogen (200 mg/mL)

Step 1: Prepare DNase reaction buffer reagents

1) Make 25mL of [1M] Tris (MW 121.14; CAS no 77-86-1)
    a. 3.0275g into 20 mL DNA-free water then adjust up to 25 mL final
    b. NOTE: If solution has a yellow color get better quality Tris

2) Make 25mL of [0.25M] $MgCl_2$ (MW = 95.21;CAS no 7786-30-3)
    a. 0.5951g into 20 mL DNA-free water then adjust up to 25 mL final

3) Make 25mL of [0.05M] $CaCl_2$ (MW = 110.98; CAS no 10043-52-4)
    a. 0.138725g into 20 mL DNA-free water then adjust up to 25 mL final

Step 2: Prepare reaction buffer (100mL):

1) Add 80mL DNA-free water to 100 mL DNA-free bottle
2) Add 1000 μL [1M] Tris (10 mM final)
3) Add 1000 μL [0.25M] $MgCl_2$ (2.5 mM final)
4) Add 1000 μL [0.05M] $CaCl_2$ (0.5 mM final)
5) Adjust solution to pH 7.6
    a. Add 5-10 DROPS of 5% HCl then confirm pH
    b. Add 3-5 DROPS [1M] NaOH then confirm pH
6) Adjust final volume up to 100 mL with MilliQ water
7) Adjust final solution to pH 7.6
    a. Add 3-4 DROPS [1M] NaOH then confirm pH

Step 3: Add 5g oyster glycogen (Sigma-Aldrich cat no. G8751-5G) to empty 50mL tube
1) Add reaction buffer up to 25 mL (approx. 20mL)
2) Agitate gently until glycogen fully dissolved then adjust volume to 25mL

Step 4: Add 25 mg DNase (1 mg/mL final; Sigma-Aldrich cat no. 10104159001) to solution
1) Mix by inversion or gentle agitation (note: do not vortex solution containing DNAse)

Step 5: Start DNase reaction by incubating (37˚C, 15 min) with gentle mixing

Step 6: Stop DNase reaction by adding 250μL [0.5M] EDTA and incubating (75˚C, 10min)

Step 7: Divide DNase-treated glycogen into 1mL aliquots and freeze (≤ 20˚C)
•Note: Remaining reaction buffer can be aliquoted (≈20mL) and frozen (≤ 20˚C)

Reagent	Price (AUD)	Unit	$ per 25mL (200mg/mL)	$ per sample [Minimum]	$ per sample [Maximum]	# Samples @ [Minimum]	# Samples @ [Maximum]
Glycogen	$131.00	5g	$131.00	$0.008	$0.031	16667	4167
DNAse	$95.50	100mg	$23.88	$0.001	$0.006		
CaCl	$86.00	100g	$0.02	$0.000	$0.000		
MgCl	$78.50	100g	$0.02	$0.000	$0.000		
[1M] Tris-HCl	$2.25	25mL	$0.09	$0.000	$0.000		
Labor (hr)	$100	2	$200	$0.012	$0.048		
TOTAL			**$355.00**	**$0.02**	**$0.09**		

Protocol 3: Silica purification

Silica matrix preparation:

1) Mix 5g Silicon dioxide (Sigma #5631) with 50mL MilliQ water
2) Allow to settle overnight[1]
3) Remove supernatant (containing fine silica particles) and add 50mL MilliQ water
4) Allow to settle overnight[1]
5) Remove supernatant (containing fine silica particles) and add 50mL MilliQ water
    a. Silica matrix ready @ [100mg/mL][2]
    b. Store at room temp for ≤ 1 year[2]

Silica-based purification of eDNA samples:

1) Add 1 volume of [3M] NaCl and 20 μL[2] silica matrix
2) Vortex and incubate at room temperature for 15min
3) Centrifuge 16,000 x g for 10 sec[2] and discard supernatant
4) Wash twice with 500 μL washing solution (add and vigorously vortex)
    a. 50% ethanol, 10 mM Tris-HCl pH 7.5, 100 mM NaCl, 1 mM EDTA[1, 2]
5) Elute DNA by resuspending silica matrix with MilliQ water equal to starting volume
6) Incubate at room temperature (≥ 20˚C) for ≥ 30 min then vortex briefly to resuspend
7) Transfer slurry onto Econospin™ column (Epoch Life Sciences, Texas USA; #1920)^
8) Centrifuge 20,000 x g for 10 sec at room temp (see Note in Step 3a)
9) Purified samples suitable for qPCR-based detection of target species*

^eDNA samples expected to contain trace copies (e.g., < 10) or intended for quantification (e.g., biomass estimation) should utilize Zymo Inhibitor Removal columns instead of Econospin™ columns ("Silica-Zymo")

*"Silica-Epoch" purified samples can exhibit 1-2 qPCR cycle shift due to trace silica carry-over (species-dependent).

[1]Huanca-Mamani, W., Rivera-Cabello, D., & Maita-Maita, J. (2015). A simple, fast, and inexpensive CTAB-PVP-Silica based method for genomic DNA isolation from single, small insect larvae and pupae. *Genetics and Molecular Research*, *14*(3), 8001-8007).

[2]Li, J. F., Li, L., & Sheen, J. (2010). Protocol: a rapid and economical procedure for purification of plasmid or plant DNA with diverse applications in plant biology. *Plant Methods*, *6*(1), 1.